Effects of a new piezoelectric device on periosteal microcirculation after subperiosteal preparation

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Abstract: INTRODUCTION: Subperiosteal preparation using a periosteal elevator leads to disturbances of local periosteal microcirculation. Soft-tissue damage can usually be considerably reduced using piezoelectric technology. For this reason, we investigated the effects of a novel piezoelectric device on local periosteal microcirculation and compared this approach with the conventional preparation of the periosteum using a periosteal elevator. MATERIAL AND METHODS: A total of 20 Lewis rats were randomly assigned to one of two groups. Subperiosteal preparation was performed using either a piezoelectric device or a periosteal elevator. Intravital microscopy was performed immediately after the procedure as well as three and eight days postoperatively. Statistical analysis of microcirculatory parameters was performed offline using analysis of variance (ANOVA) on ranks ($p<0.05$). RESULTS: At all time points investigated, intravital microscopy demonstrated significantly higher levels of periosteal perfusion in the group of rats that underwent piezosurgery than in the group of rats that underwent treatment with a periosteal elevator. CONCLUSION: The use of a piezoelectric device for subperiosteal preparation is associated with better periosteal microcirculation than the use of a conventional periosteal elevator. As a result, piezoelectric devices can be expected to have a positive effect on bone metabolism.

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Effects of a new piezoelectric device on periosteal microcirculation after subperiosteal preparation☆

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A B S T R A C T

Introduction: Subperiosteal preparation using a periosteal elevator leads to disturbances of local periosteal microcirculation. Soft-tissue damage can usually be considerably reduced using piezoelectric technology. For this reason, we investigated the effects of a novel piezoelectric device on local periosteal microcirculation and compared this approach with the conventional preparation of the periosteum using a periosteal elevator.
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Introduction

The periosteum is a membrane that consists of connective tissue and covers the bone. Morphologically, the periosteum can be divided into three zones, each of which contains highly specific cells. The inner zone is the osteogenic layer that contains cells similar to those of the endosteum. Among these cells are mesenchymal stem cells, osteoprogenitor cells, active and resting osteoblasts, and/or active and resting osteoclasts. The middle zone is a translucent layer that is characterized by a large number of microvessels. The outer zone is a typical fibrous layer that contains collagen fibers (Squier et al., 1990).

The specific structure of the periosteum is seen not only in children but also in adults and allows bones to remodel themselves over time, for example during bone fracture healing (Kowalski et al., n.d; Landry et al., 2000; Ruecker et al., 1998). Periosteal cells play a major role in the supply of blood to the bone. The importance of intact periosteal tissue is underlined by the substantial contribution of periosteal blood cells to the supply of blood to the cortical bone (70–80% of arterial supply and 90–100% of venous return) when compared to intraosseous blood vessels (Chanavaz, 1995). The periosteum is closely attached to the bone by collagen fibers in the bone matrix and by hemidesmosomes (Junqueira et al., 1996). Surgical procedures, especially those directly involving bone, often have adverse effects on the osteogenic potential of the periosteum since they are associated with the detachment of periosteal tissue from the bone. Periosteal damage can either be caused by the deliberate separation of the periosteum from the bone during surgery or it can be the result of a disease or trauma.

The preparation of the periosteum is a routine procedure in trauma surgery, reconstructive surgery and especially dentoalveolar surgery (Flores-de-Jacoby, 1987; Harrison and Jurosky, 1991; Kramper et al., 1984; Lutz and Schlegel, 2000). It is commonly performed using a periosteal elevator that is used for manually lifting and separating periosteal tissue from the bone. This procedure causes damage to the morphological structure of the periosteum and especially to the cells of the osteogenic layer. The result is a complete or partial loss of periosteal function (Mercurio et al., 2012; Schaser, 2003). It is currently impossible for surgeons to prepare the periosteum between the osteogenic layer and the underlying bone in such a way that the periosteum remains intact. The use of a periosteal elevator leads to the disruption of periosteal integrity

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and the separation of the periosteum from the bone as a result of a process that is mainly mechanical in nature. The destruction of the connection between bone and periosteum damages the regenerative cells of the periosteum and reduces their osteogenic potential (Bilkay et al., 2000; Li et al., 2004; Svindland et al., 1995). Successful osteoinduction and osteoconduction, however, require the preservation of cell vitality in the periosteum (Schaser, 2003). Periosteal cells provide nutrition to the underlying bone by free diffusion. Adequate functioning of the periosteum is of far greater importance to patients who have underlying diseases such as diabetes mellitus or undergo tumor treatment and receive chemotherapeutic agents than it is to healthy people since the periosteum plays an important role in promoting rapid bone healing. If these patients undergo surgery involving bone, particular care must be taken to cause no damage or as little damage as possible to the periosteum with a view to ensuring subsequent bone healing without dehiscences or necrosis (Claes and Heigele, 1999). If the bone is damaged without compromising local periosteal microcirculation, good bone healing can be expected. If, by contrast, local periosteal microcirculation is compromised, the regenerative potential of the periosteum will be reduced. Good periosteal microcirculation is of paramount importance for bone modeling (Mercurio et al., 2012). In the literature, there is only a paucity of chronic studies on periosteal perfusion during and after subperiosteal preparation.

Whereas piezoelectric ultrasonic instruments have been available since 1988, devices utilizing the piezoelectric effect have been used for medical purposes only since 1998. Applications of piezoelectric devices include hard-tissue surgery, periodontal surgery, the removal of impacted teeth, apical surgery (Vercellotti, 2004; Vercellotti et al., 2001), and bone expansion (Metzger et al., 2006; Schlee et al., 2006).

The piezoelectric effect is based on physical interactions in crystalline materials. The application of an electric field creates nanoscale deformations in a crystal. This dynamic effect can be used to transform longitudinal or transverse movements of a ferroelectric material into a surgical cutting action. Piezoelectric devices are operated at different frequencies depending on the density of the tissue to be cut.

The tip of the ultrasonic device vibrates within a range of 20–200 μm at a frequency of 20,000 Hz. Piezoelectric devices are permanently cooled with sterile physiological saline during use so that heat-induced trauma can be largely ruled out (Bacci et al., 2010; Berengo et al., 2006) and the risk of bacterial contamination is minimized.

The essential difference between piezoelectric devices and conventional preparation instruments is that piezoelectric devices operate in a tissue-specific manner. Every tissue has a specific frequency range at which it can be cut. A piezoelectric device can therefore cut a specific type of tissue without causing damage to adjacent tissues. Damage to the soft tissues (e.g. nerves) that surround bone, for example, is caused only at frequencies above 50 kHz (Vercellotti, 2000; von See et al., 2011). In addition, piezoelectric devices have the advantage that they cause minimal bleeding when they are used to cut bone. The extent to which piezoelectric devices adversely affect periosteal microcirculation has not yet been investigated. While there are a few studies that address the behavior of bone when it is being cut by piezoelectric devices, there are no studies that examine local microcirculation within the periosteum during and after the cutting operation. We conducted this study in order to investigate the effects of piezoelectric surgery on local periosteal microcirculation and compared the use of a piezoelectric device and a conventional periosteal elevator for the preparation of the periosteum.

Material and methods

Laboratory animals

All procedures were approved by the responsible authority (Ref. 12/0861) and were performed in accordance with the German Animal Protection Act and the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The study involved 20 adult male Lewis rats with a body weight between 300 g and 330 g (Harlan-Winkelmann, Borchen, Germany). The rats were housed singly in cages at a room temperature of 22–24 °C and a relative humidity of 60–65% with a 12-hour day/night cycle. They received water and dry food (Altromin, Lage, Germany) ad libitum during the entire investigation.

Study design and experimental groups

Microcirculatory parameters were assessed on day 0 immediately after subperiosteal preparation with the different instruments and on days 3 and 8 after the procedure. The experiments were performed on the basis of a model established by Stuehmer et al. (2009).

The rats (n = 20) were divided into two experimental groups.

Group 1 n = 10, subperiosteal preparation with a periosteal elevator, intravital microscopy

Group 2 n = 10, subperiosteal preparation with a piezoelectric device, intravital microscopy

Procedures

The animals were anesthetized using an intraperitoneal injection of ketamine (Ketavet®, 75 mg per kg bodyweight, Parke-Davis, Germany) and xylazine (Rompun®, 25 mg per kg bodyweight, Bayer HealthCare, Germany). A surgical blade was used to make an incision through the skin and periosteum in the occipital region in order to expose the calvaria. Depending on the group, either a periosteal elevator or a piezoelectric device was used for the preparation procedure. The skin was then repositioned and secured in place with sutures (Ethicon Vicryl® sutures 4-0, Johnson & Johnson, Germany). The procedure took approximately 10 min. Intravital microscopy was performed subsequently. Periosteal vascularization was analyzed by intravital microscopy on the following days at the time points indicated above. Every microscopic examination took approximately 30 min.

Intravital fluorescence microscopy of the periosteum

Under anesthesia with intraperitoneal ketamine (Ketavet®, 75 mg per kg bodyweight) and xylazine (25 mg per kg bodyweight), intravital fluorescence microscopy was performed immediately after the preparation of the periosteum and on days 3 and 8 after the procedure. Fluorescein–isothiocyanate-labeled dextran (FITC-dextran, molecular weight: 150,000 Da, Sigma, Taufkirchen, Germany, 5% in 0.9% NaCl solution, 0.1 ml) was injected into the tail vein of each animal for contrast enhancement of blood plasma. This technique permitted the imaging of microcirculation. All examinations were recorded on-line using a highly sensitive video camera and quantitatively analyzed (off-line) with computer assistance at a later time in order to minimize examination times.

Reflected light fluorescence microscopy was performed using a Zeiss Axiotech microscope (Zeiss, Oberkochen, Germany) at 20× magnification. A blue filter block (450–490 nm) permitted the visualization of blood plasma. Microscopic images were recorded using a highly sensitive video camera (FK 6990 IQ-S, Pieper, Schwerte, Germany) and transferred to a DVD system (LQ-MS 800, Panasonic, Hamburg, Germany) for off-line evaluation.

Image analysis

Computer-assisted quantitative image analysis was performed off-line using CapImage image analysis software (Zeintl, Heidelberg, Germany). Functional capillary density, microvessel diameters and volumetric blood flow were determined in the venules. Functional vessel density was assessed on the basis of the length of perfused microvessels per observation area. Diameters (d) were measured perpendicular to

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the vessel path and are expressed in mm. Volumetric blood flow was calculated using the formula: $\pi \times (d/2)2 \times v/K$, where K represents the Baker–Wayland factor to correct for the parabolic velocity profile in microvessels with a diameter >20 μm (Baker and Wayland, 1974).

Statistical analysis

Normal distribution and homogeneity of variance were assessed. Results are expressed as means and standard errors of measurement (SEM). Differences between groups were evaluated with a one-way analysis of variance (ANOVA) on ranks. Differences within groups were also analyzed by ANOVA. Student–Newman–Keuls or Duncan post-hoc tests were used to isolate specific differences. A p-value <0.05 was considered significant. Data was collected and analyzed using Microsoft Office Excel 2007 and IBM SPSS (Statistics 21, IBM Deutschland GmbH, Germany).

Results

Intravital fluorescence microscopy

Periosteal microcirculation was imaged in detail using intravital fluorescence microcopy. The group of rats whose periosteum had been prepared with a piezoelectric device was compared with the group of rats whose periosteum had been prepared with a periosteal elevator.

The periosteal elevator group had a significantly lower functional capillary density than the piezoelectric device group at all time points investigated. Postoperatively, both groups showed a considerable increase in functional capillary density, which, however, was always lower in the periosteal elevator group than in the piezoelectric device group (Fig. 1). Means and standard deviations are given in Table 1.

Microvascular red blood cell velocity was significantly lower in the periosteal elevator group than in the piezoelectric device group. On day 0, red blood cell velocity was 0.31 mm/s (±0.12) in the periosteal elevator group and 0.69 mm/s (±0.43) in the piezoelectric device group. During the following 8 days, the red blood cell velocities in the periosteal elevator group became more similar to those measured in the piezoelectric device group. At no time point, however, were red blood cell velocities in the periosteal elevator group as high as or higher than those in the piezoelectric device group. Microvascular red blood cell velocities were almost constant in the piezoelectric device group and increased only moderately in the periosteal elevator group during the observation period (Fig. 2). Means and standard deviations are shown in Table 2.

On day 0, only vessels with a small diameter were found in the periosteal elevator group. Vessel diameters considerably increased during the observation period. In the piezoelectric device group, the diameters of perfused microvessels were considerably larger than those in the other group at all time points and changed only mildly during the observation period (Fig. 3). Means and standard deviations are given in Table 3.

Table 1
Means and standard deviations (SD) for microvessel density.

Days after surgery	Periosteal elevator group [cm/cm²] (mean/SD)	Piezoelectric device group [cm/cm²] (mean/SD)
0	22.73 ± 13.98	80.69 ± 17.66
3	31.00 ± 18.67	68.96 ± 20.31
8	120.15 ± 99.31	127.96 ± 36.56

Discussion

In the study presented here, a novel device for the preparation of the periosteum was compared with a conventional periosteal elevator in an animal model.

Microvascular perfusion of different types of tissues can be investigated in vivo by a variety of methods such as laser Doppler flowmetry and polarographic oxymetry (Baker and Wayland, 1974; Kowalski et al., n.d; Menger et al., 1992). The main disadvantage of these methods is that tissue perfusion can be imaged only indirectly and that no information about the perfusion of individual microvessels can be obtained.

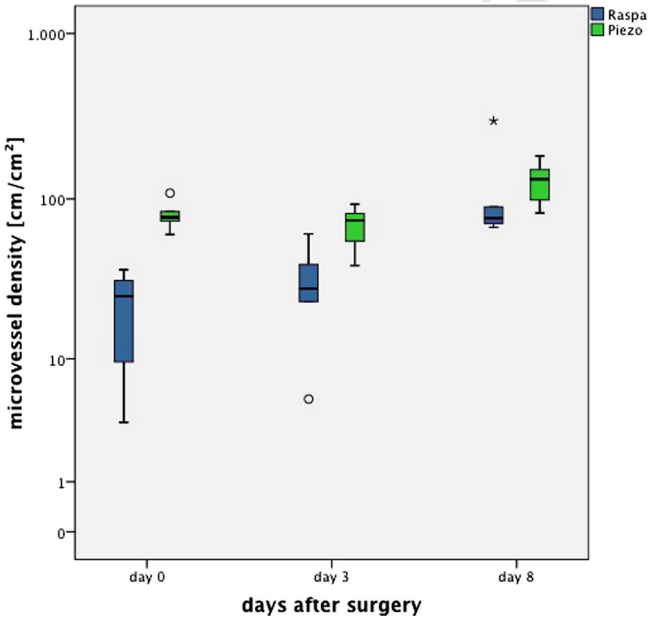

Fig. 1. Microvessel densities (logarithmic scale) at different time points (days 0, 3 and 8). Outliers that are more than 1.5 times the length of the box below the 25th percentile or above the 75th percentile are represented as circles and outliers that are more than 3 times the length of the box below the 25th percentile or above the 75th percentile are represented as asterisks.

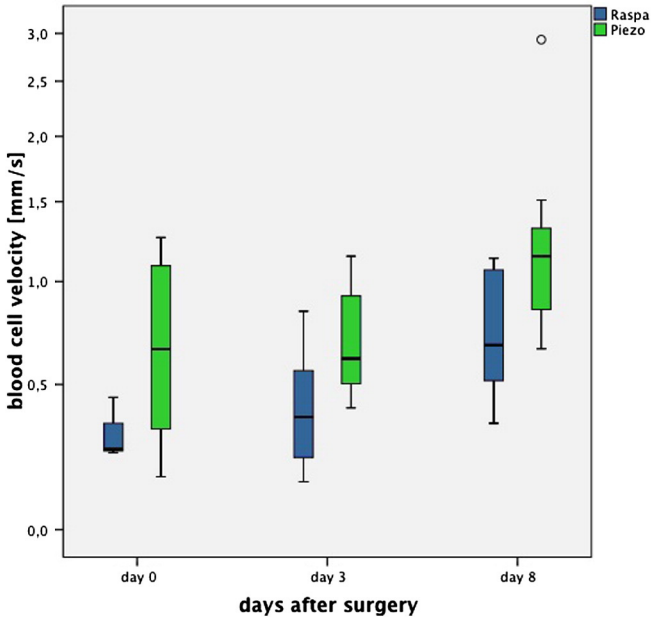

Fig. 2. Blood cell velocities on days 0, 3 and 8. For a better view, the y-axis uses a logarithmic scale. Outliers that are more than 1.5 times the length of the box below the 25th percentile or above the 75th percentile are represented as circles and outliers that are more than 3 times the length of the box below the 25th percentile or above the 75th percentile are represented as asterisks.

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Table 2
Means and standard deviations (SD) for blood cell velocity.

Days after surgery	Periosteal elevator group [mm/s] (mean/SD)	Piezoelectric device group [mm/s] (mean/SD)
0	0.31 ± 0.12	0.69 ± 0.43
3	0.42 ± 0.25	0.72 ± 0.31
8	0.75 ± 0.34	1.30 ± 0.78

Table 3
Means and standard deviations (SD) for microvessel diameter.

Days after surgery	Periosteal elevator group [μm] (mean/SD)	Piezoelectric device group [μm] (mean/SD)
0	8.94 ± 7.40	17.72 ± 5.36
3	14.57 ± 6.37	16.78 ± 6.71
8	13.86 ± 2.40	17.39 ± 5.25

By contrast, intravital microscopy offers the possibility of studying the perfusion of individual microvessels even over a prolonged period of time (Baker and Wayland, 1974; Vollmar et al., 1996). This method has been shown to be suitable for investigating periosteal perfusion in other studies (Gülnahar et al., 2013; von See et al., 2010). The technique that was used in the study presented here, i.e. intravital fluorescence microscopy, is an established method and has been used by Menger et al. (1992) in the past twenty years for a wide variety of investigations. Although the transferability of the results of these studies to physiological and pathological processes in humans is limited, findings for a specific species can be directly compared and provide a basis for reliable conclusions.

We determined functional capillary density, blood flow within microvessels and the diameters of microvessels in the periosteum in order to investigate whether a piezoelectric device causes less irritation to microvessels than a conventional periosteal elevator.

Our results show that the use of the piezoelectric device for the preparation of the periosteum was associated with a considerably higher post-procedural periosteal blood flow than the conventional method with a periosteal elevator. Several studies reported that piezosurgery is an atraumatic process that causes only minimal tissue damage (Gülnahar et al., 2013). The study presented here confirms this finding for subperiosteal preparation. One possible explanation is that the use of a piezoelectric device leads to the formation of fewer microthrombi during subperiosteal preparation than a periosteal elevator. Schaser et al. (Schaser, 2003), for example, showed that trauma to the periosteum resulted in the formation of microthrombi and disturbances of perfusion within the periosteum.

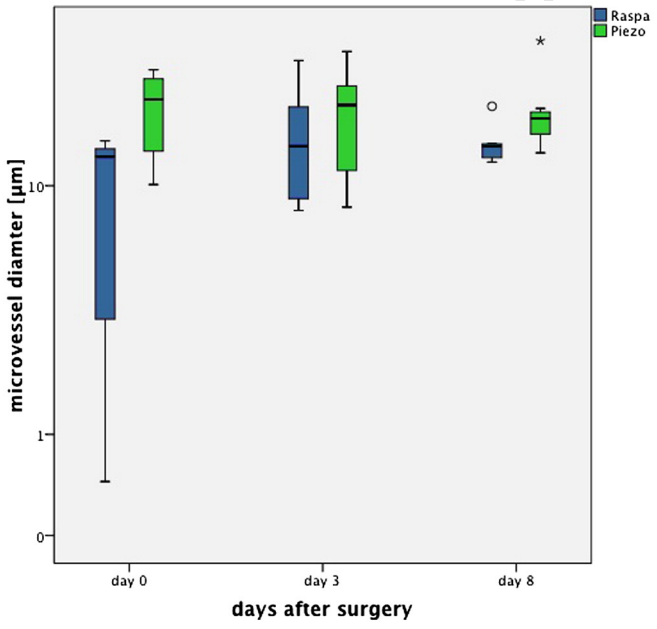

Fig. 3. Microvessel diameters on days 0, 3 and 8. For a better view, the y-axis uses a logarithmic scale. Outliers that are more than 1.5 times the length of the box below the 25th percentile or above the 75th percentile are represented as circles and outliers that are more than 3 times the length of the box below the 25th percentile or above the 75th percentile are represented as asterisks.

Functional capillary density was significantly higher after preparation with a piezoelectric device. As a result, a considerably higher number of perfused vessels were available for periosteal supply. In addition, the piezoelectric device was associated with a significantly higher microvascular blood flow than the periosteal elevator.

Vessel density in the periosteum plays an important role in the supply of blood to the bone (Chanavaz, 1995). Every surgical procedure that leads to subperiosteal exposure compromises microcirculation (Kowalski et al., n.d). For this reason, surgeons always attempt to preserve the integrity of the periosteum as far as possible during orthopedic and trauma surgeries (Gautier and Ganz, 1994).

Conclusions

The results reported here show that the use of a piezoelectric device for the preparation of the periosteum has considerable advantages. Further studies are required to investigate possible positive effects on bone remodeling in patients who have comorbidities and, for example, are treated with bisphophonates, chemotherapeutic agents, or other medications.

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